REACTIONS OF AMŒBA PROTEUS TO FOOD.

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In material taken from a pond southwest of the university we found great numbers of *Amœba proteus*. These animals appeared in a brown scum at the surface of the aquarium. The *Amœba* is one of the simplest animals and hence is an attractive form in which to study the phenomena of life. The simplest vital phenomenon—movement—is of course involved in the food reactions of *Amœba*. Rhumbler and others once held that the movement of *Amœba* could be explained by means of variation of surface tension upon the body of the animal. Jennings was the first to attack this theory. He demonstrated the currents of the protoplasm on the surface of *Amœba* by causing soot to adhere to the surface of the animal. These experiments showed that surface tension could not explain these currents and therefore could not explain the movement of *Amœba*. Later Dellinger ('06) likewise showed that surface tension could not explain this movement, for he saw that in advancing an *Amœba* threw out a pseudopodium, the end of which it fixed by adhesion to the substratum and then through the contraction of this fixed pseudopodium the body was dragged to the point of attachment to the substratum. Rhumbler ('05) according to his reviewer in answer to Jennings' criticism pointed out that in his work "it (Rhumbler's surface tension theory of movement) is not dependent on the movements termed 'Föntanen-strömung,' whose existence Jennings calls in question. These movements, though not frequent, certainly do occur in some *Amœbæ:* it is not their unconditional necessity, but their theoretical value as a starting point, which accounts for their occupying the chief place in the author's theories. It is not claimed that there is more than a parallel value or 'convergence' shown in the comparative experiments with organic and inorganic mechanics; the chemistry in both is fundamentally different. It is possible that in

the *Amœba* resides a 'Miniaturpsyche'—an energy absent in the inorganic." Thus the simplest phenomenon of life in one of the simplest animals has not been reduced to terms of physics and chemistry.

Despite this fact there is much, as yet, said concerning the physical and chemical explanation of the food-reactions of *Amœba*. For instance Hegner ('10) says in speaking of the food reaction of *Amœba* that "this apparent choice of food may be due to ordinary physical laws of fluids." Likewise Calkins ('09) says: "While most of the actions of protozoa are reactions to external stimuli, many are combinations of reactions which do not lend themselves to analysis. Such, for example, is the apparent choice of food or of building material for shells and tests, or the complex reactions that are frequently involved in the avoidance of some obstruction. Many of these so-called conscious acts can be explained by the ordinary physical laws of fluids."

At a more recent date McClendon ('12) showed that by a disturbance of the electrical polarization the surface tension would be modified and adds: "We might conclude therefore that the low surface tension of the *Amœba* is caused by electric polarization, due to the production of some metabolic electrolyte whose anions cannot escape; and that strong stimulation causes increased permeability and hence disappearance of the electrical polarization.

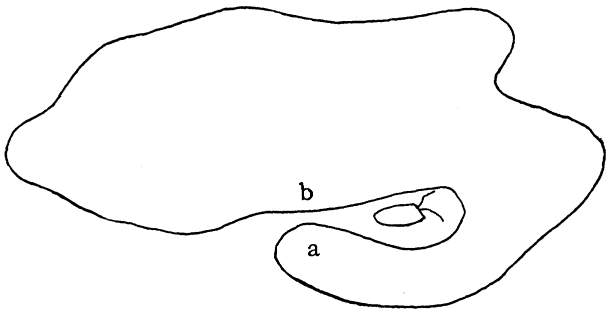
"This would explain all negative tropisms of the *Amœba*. The surface tension of the portion most strongly stimulated is increased, and the *Amœba* flows away from the stimulus.

"In order to explain positive tropisms we would have to make another assumption. If the stimulus did not react directly on the plasma membrane, but penetrated the *Amœba* and acted on the protoplasm, and increased the production of the metabolic product producing polarization of the plasma membrane, it would thereby decrease the surface tension. The local decrease in surface tension would cause the *Amœba* to flow toward the source of the stimulus, just as the quicksilver drop in dilute HNO_3 flows toward potassium bichromate in Bernstein's experiment."

The food-reaction of *Amœba* involves movement. It is however, a more complex phenomenon than mere movement. "The capture and ingestion of food, in its simplest form, occurs in the group of Rhizopoda, where, as in *Amœba proteus*, any part of the body can act as a mouth. In this form pseudopodia are pushed out toward the victim (a flagellate, ciliate, minute plant form of any kind, or even a higher animal, such as a rotifer or worm) and entirely surround it, together with a certain amount of water, thus forming a gastric vacuole, or an improvised 'stomach'" (Calkins '01). This description gives the details as generally given for the ingestion of food.

So far as we have been able to determine no departure from this simple method of capturing food by an *Amœba proteus* has been described. Jennings observed an *Amœba* persistently push a spheroidal *Euglena* cyst from place to place in an effort to ingest it. He also observed an *Amœba proteus* ingest repeatedly a smaller specimen of *Amœba proteus*, the latter each time breaking through the protoplasmic wall of its captor. But even in these most interesting instances the prey was taken into the body by the ingesting protoplasm flowing about each side of the prey with equal velocity. On September 25, Mr. F. L. Kline, Mr. W. A. Williams, Mr. J. P. Williams and Mr. R. T. Scott called the attention of one of us to an *Amœba proteus* that was sending a pseudopodium out from the side of its body posteriorly in such a way as to surround a quiet *Chilomonas paramœcium*. In this case it was remarkable that the entrapping protoplasm was flowing about but one side of the prey, the side of the *Amœba's* body furnishing one wall of the forming food vacuole. Later Mr. Scott observed that the food vacuole was completed through the fusion of the end of the pseudopodium and the side of the body (text-figure 1). September 26, Mr. E. M. Baker called our attention to an *Amœba proteus* that had a *Chilomonas paramœcium* lying between two pseudopodia as indicated in Fig. 1, *a*. He had not observed where the contact with the prey had been made, but it was evident that the two pseudopodia were closing up behind the *Chilomonas paramœcium*. We then called the attention of Mr. C. A. Amos, Mr. H. H. Buehler, and Mr. A. H. Brewster to the specimen. As we took turns with these

men in observing it the left pseudopodium alone grew. As it elongated it travelled along the inner side of the right pseudopodium to its base. At the base of the right pseudopodium the growing left one turned along the surface of the body and then travelled "anteriorly" until the prey was entwined by it. When thus the enclosing space had been greatly reduced the left pseudo-



TEXT-FIGURE 1. Outline of an *Amœba proteus*, showing the manner in which the pseudopodium *a* developed along the outer side of a *Chilomonas*. Eventually the apex of the developing pseudopodium, *a*, fused with the body at *b*. In this manner the *Chilomonas* was taken into the body of the *Amœba*. (The position of the axis of the *Chilomonas* was not observed. The point of contact of the *Chilomonas* with the surface of the *Amœba* was likewise not noted.)

podium fused along its two margins at the place indicated in Fig. 1, *e* by a broken line.

These observations led us to make further studies on the food reactions of *Amœba proteus*. An *Amœba proteus* with two pseudopodia projecting and flowing for the most part into the larger one came in contact with a quiet *Chilomonas paramœcium* (Fig. 2). The *Chilomonas paramœcium*, thus disturbed, retreated to the position indicated by the finely stippled outline (Fig. 2). Again the pseudopodium came in contact with the *Chilomonas* and the latter then retreated to the position indicated by the darkest contour. As the *Chilomonas* lay in this position the *Amœba proteus* approached it and this time without disturbing the flagellate sent protoplasmic processes about its sides until the prey was enclosed within a food vacuole. This observation does not depart greatly from what has been generally described as the usual reaction of *Amœba proteus* to food.

The next observation, however, is quite unusual. The *Chilomonas paramœcium* in this case ran into the side of an *Amœba proteus* that was "flowing into" a curved pseudopodium. In this case the large pseudopodium flowed back along the outer side of the *Chilomonas* until the tips of the two pseudopodia fused (Fig. 3).

We have seen three instances of a *Chilomonas paramœcium* and one instance of a diatom entering the *narrow* angle between two pseudopodia and coming in contact with the ectoplasm at the apex of this angle. In all these cases the reactions were analogous. The specimen represented in Fig. 4 had a *Chilomonas paramœcium* swim into the narrow angle between two pseudopodia and which made repeated contacts at *a*. The response to this stimulus at *a* resulted in the formation of pseudopodia behind the *Chilomonas* at *b* and *c*.

In contrast with the last observations is one made upon a specimen that had two *widely* diverging pseudopodia. The general movement of the body was in the larger pseudopodium. A *Chilomonas paramœcium* came in contact with the middle of the mesial surface of the smaller pseudopodium. The flagellate made a single impact at this point and lay in contact with the ectoplasm. In response to this contact the *Amœba proteus* sent out a third protoplasmic process from the apex of the angle between the first two pseudopodia (Fig. 5, *a*), thus placing the object of prey in a narrow angle between two pseudopodia. Before the end of pseudopodium *a* reached the level of the end of its neighbor it changed its course so as to flow behind the *Chilomonas*. At the same time the original pseudopodium sent out a secondary one (Fig. 5, *b*) below its apex to meet the other enclosing pseudopodium. In this way the *Chilomonas* was enclosed in a food vacuole of about the usual size.

Other conditions may arise with reference to the capturing of the *Chilomonas paramœcium* by the *Amœba proteus*. *Chilomonas* sometimes seems quite indifferent to the contact of the *Amœba proteus*. On September 30, a specimen of the latter was observed which came in contact with a flagellate at the apex of its more active pseudopodium (Fig. 6, *a*, 1.) As the pseudopodium grew the *Chilomonas* glided along its surface to the

position 2 in Fig. 6, *a*. When the flagellate was left a little farther beyond the apex of the growing pseudopodium the streaming movement of the latter changed its course so as to cause the pseudopodium to send a process or secondary pseudopodium off behind the quiet *Chilomonas*. This process or secondary pseudopodium, through a streaming at right angles to the larger one, grew somewhat larger, then its course became oblique and it traveled like a wave posteriorly along the mesial surface of its parent pseudopodium. This wave-like movement of the secondary pseudopodium carried the yet quiet *Chilomonas* down to the apex of the angle between the two original pseudopodia. Thus the prey was brought into a relatively small space, which through the fusion of the tip of the secondary pseudopodium and a region near the base of one of the original pseudopodia, became a food vacuole (Fig. 6, *a*, *b*, *c* and *d*).

Perhaps the most interesting condition we have seen presented to the ingenuity of the *Amœba proteus* is to be seen in the following observation. The *Amœba proteus* was traveling along the line of a large pseudopodium when it came in contact with a quiet *Chilomonas paramœcium*. At first the *Amœba proteus* seemingly did not react but simply pushed the *Chilomonas* to one side (Fig. 7, 2). At this point, however, the *Amœba proteus* protruded a small pseudopodium from the apex of the larger one which proceeded around the *Chilomonas* (Fig. 7, *a*). At the same time it threw out a secondary pseudopodium from the body of the larger one some distance below the *Chilomonas* (Fig. 7, *b*). It, however, withdrew this secondary pseudopodium at *b* and threw out another one (Fig. 7, *c*), closer to the *Chilomonas* which immediately proceeded to meet the first enclosing pseudopodium, *a*.

After the extreme variability of the *Amœba's* reactions to food as seen in these observations one is not justified in saying as does Hegner ('10), "This apparent choice of food may be due to the ordinary physical laws of fluids." In the first place as many if not more *Chilomonas paramœcia* were rejected as were accepted by the *Amœbas*. Frequently a *Chilomonas paramœcium* would come in contact with the "anterior end" or side and then remain by the *Amœba* without being ingested. If this were a process involving the "ordinary physical laws of fluids" there would

be no rejection of food when it presented itself in a manner favorable for acceptance. Nor does it follow that when an *Amœba* has rejected food that this rejection is final. On September 26, Mr. L. Grady Burton saw an *Amœba proteus* which was flowing along the line of a large pseudopodium come in contact with a quiet *Chilomonas paramœcium.* The *Amœba proteus* twice touched the *Chilomonas* and each time withdrew its pseudopodium. The *Amœba proteus* then moved around and by the *Chilomonas.* It now lay opposite a side which was at right angles to the one first encountered. The *Amœba* then sent out a pseudopodium at right angles to the first one and again touched the *Chilomonas.* It rejected the flagellate again but on touching it the second time in this new position the *Amœba proteus* ingested the *Chilomonas.*

These reactions too show a departure from the description given by Jennings ('06). "The essential features of the food reaction seem to be the movement of the *Amœba* toward the food body (long continued, in some cases), the hollowing out of the anterior end of the *Amœba*, the sending forth of pseudopodia on each side of and above the food, and the fusion of the free ends of the pseudopodia thus enclosing the food, with a quantity of water. The reaction is thus complex; at times, as we have seen, extremely so."

As we have been able to observe the reactions of the *Amœba proteus* are not always "the hollowing out of the anterior end of the *Amœba*, the sending forth of pseudopodia on each side of and above the food, and the fusion of the free ends of the pseudpodia"; but the reaction is a variable one and may arise from the side of the *Amœba's* body and involve primarily but a single pseudopodium.

Moreover the reaction of an *Amœba proteus* to food is in each case determined by the conditions presented at the time of contact. Some of these conditions are:

1. The metabolic condition of the *Amœba proteus*, demanding or not demanding food.

2. The form of the *Amœba's* body at the time the food is presented to it with reference to (*a*) the amount of water that may enter a food vacuole, and (*b*) the possible retreat of the object of prey.

So far as our observations are concerned little can be said about the first condition. The relative number of food vacuoles did not seem to make any marked difference in the conduct of the *Amœba* with reference to food. It was not in our power in any way to determine whether the metabolic conditions of the *Amœba* demanded food or not.

Our observations are chiefly concerned with the manner in which the *Amœba* met the conditions of not getting too great an amount of water into the food vacuole and of not letting the prey escape. We can readily understand how by dilution of ferments a relatively large quantity of water in a food vacuole might present a condition to the *Amœba* in taking in food. The amount of water, therefore, that may enter a food vacuole is one of the dominate factors in controlling the way in which an *Amœba* reacts to the prey.

This condition we infer was the primary one determining the action of the *Amœba* represented in Fig. 1. Here the object of prey when first seen lay out away from the surface in a wide angle between two pseudopodia. The stimulus of its contact (not observed by any of us) resulted in the pseudopodia bending their tips towards each other. Had these tips fused the basis of a very large food vacuole would have been formed. No fusion took place here. In the manner described above the one pseudopodium was wrapped about the *Chilomonas* until a relatively small space was enclosed over which an ectoplasmic film arched leaving only a small bilobed aperture (Fig. 1, *e*, *ap*). Out of this small bilobed aperture water was apparently forced, for the enclosure became gradually smaller and after it was reduced to the size of the food vacuole usually encountered the bilobed opening was closed through the convergence and fusion of its lips. After this pore was closed the endoderm also came to lie over the upper side of the food vacuole. In this rather remarkable manner the prey was not allowed to escape and a food vacuole with a relatively small amount of water was formed.

The reaction of the *Amœba* represented in Fig. 2 is like that usually described for the conduct of an *Amœba* with reference to food. Here the amount of water that is to enter the food vacuole is not so great a conditioning factor but the reaction is rather

conditioned by the great chances afforded for the escape of the prey. So after disturbing the prey twice and causing it to retreat as often along the projected axis of the pseudopodium the *Amœba* did not disturb the prey but sent about it pseudopodia which eventually enclosed the quiet *Chilomonas*. This is of further interest in demonstrating that the reaction of *Amœba* to food is not a fixed one for twice the *Chilomonas* was rejected and the third time deliberately accepted.

In contrast with this reaction is that of the *Amœba* shown in Fig. 3. Here a *Chilomonas*, after making contact with the ectoplasm of the *Amœba*, lay in between two pseudopodia, one of which presented an inwardly directed surface and the other an outwardly directed surface. If the disturbed prey were to strike the former it would be deflected towards the fundus of the interpseudopodial space, whereas if the disturbed prey were to strike the latter it would be deflected away from the space which is to form the basis of a food vacuole. Thus in this case the possible retreat of the prey may be the chief condition to be met.

When an object of prey entered a narrow angle between two pseudopodia the possible retreat of the prey again became the principal conditioning factor. If the *Amœba* had reacted at the point of contact by sending out secondary pseudopodia towards each side of the prey it would have crowded back the prey and thus result in its escape. To prevent this escape the *Amœba* sent secondary pseudopodia behind the *Chilomonas paramœcium* as described for Fig. 4 on page 415.

Further it is interesting to note that in this case the stimulus was encountered at *a* and the reaction took place at *b* and *c* (Fig. 4). Thus we see that here it is not as Jennings ('06) says in reference to locomotion that "it is primarily the part stimulated that responds." For in this reaction it was not the part immediately stimulated but the parts that could most advantageously respond that did so.

Where it was more advantageous for another part or parts to respond we find that they responded. Take for example the case in which the object of prey entered a broad angle between two pseudopodia as described on page 415 for Fig. 5. If the *Amœba* had reacted in the manner just described it would have

been easier for the prey to escape and the food vacuole would have contained a large quantity of water. For these and probably other unknown reasons the *Amœba* reacted by sending out a secondary pseudopodium which placed the prey in a narrow angle and then ingestion took place practically the same way as described above for the reaction to food in a narrow angle (Fig. 5).

Before leaving observations 4 and 5 a useful comparison should be made. In the reaction of the *Amœba* represented in Fig. 4 the suggestion might be raised that pseudopodia grew with equal velocity at *b* and *c* because of more or less equal stimuli caused by vortices in the wake of the prey. But in answer to this suggestion we have the reaction of the *Amœba* in Fig. 5. Here we see the contact was made obliquely to the surface and that the larger pseudopodium arose at a point most remote from the path of the prey. *So the reaction was greatest where the stimulus was weakest but where the conditions demanded greatest reaction.*

When the *Chilomonas paramœcium* in Fig. 6 was left in position 2 it lay at a point on the surface which would not direct its possible movements towards the fundus of the space between the two pseudopodia. So, as if in order to prevent its direct retreat from this space, a secondary pseudopodium grew up behind the prey. The manner in which the secondary pseudopodium travelled as a wave along the mesial surface towards the fundus of the interpseudopodial space met the conditions determined by the possible escape of the prey and the usual or maximum size of a food vacuole.

The second relatively large pseudopodium in this case seems to have been a determining structure in this reaction. The position of the *Chilomonas* too may have been a factor in it, for in these two respects the *Amœbas* shown in Figs. 6 and 7 differ. In the latter there is no second pseudopodium to coöperate with the first and the axis of the body of the *Chilomonas* shown in Fig. 6 lies more or less parallel to the surface of the pseudopodium whereas in the *Amœba* shown in Fig. 7 the *Chilomonas* in position 2 lies at right angles to the surface of the pseudopodium. Thus the *Chilomonas* in the latter instance has all paths for retreat open except that towards the surface of the pseudo-

podium. Hence the possibility of escape of the prey again becomes the conditioning factor in the reaction, which is met in the manner described on page 416.

In the *Amœba* which presented the reaction shown in Fig. 7 we again see that it is not the part stimulated that primarily reacts nor is the response for a given stimulus a fixed one. The stimulus in this case was made at the point *d* and at first the tip of the pseudopodium *a* grew along the far side of the prey while a smaller pseudopodium arose at *b*. Later this first reaction was greatly modified to better meet the involved conditions. This modification was accomplished through a negative response at *b* and a positive response at *c*. This reaction, so modified, was a better one in so far that it made the escape of the *Chilomonas* less probable. Thus we have an example of the *Amœba proteus* attaining its end through trial and error since the reaction to a given stimulus was modified with reference to more readily realizing the object of the reaction.

Conclusions.

1. The reactions of *Amœba proteus* are highly variable in reference to: (*a*) power of accepting or rejecting food; (*b*) method of ingesting food.

2. In all this variability of reaction there appears no haphazardness. The reactions are not automatic responses. Each reaction is a response made to suit the peculiar conditions presented at the moment of accepting or rejecting the food; and if not well suited to meet these conditions, it is modified with reference to better meeting them. Thus in each reaction there is evidence of purposiveness.

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[1] The same day that the page proof of this article came to the authors Prof. F. J. Wright sent us a reprint of "Daily Life of Amœba Proteus" by David Gibbs, Clark University. No reference has been made to this important paper in our paper. Authors.

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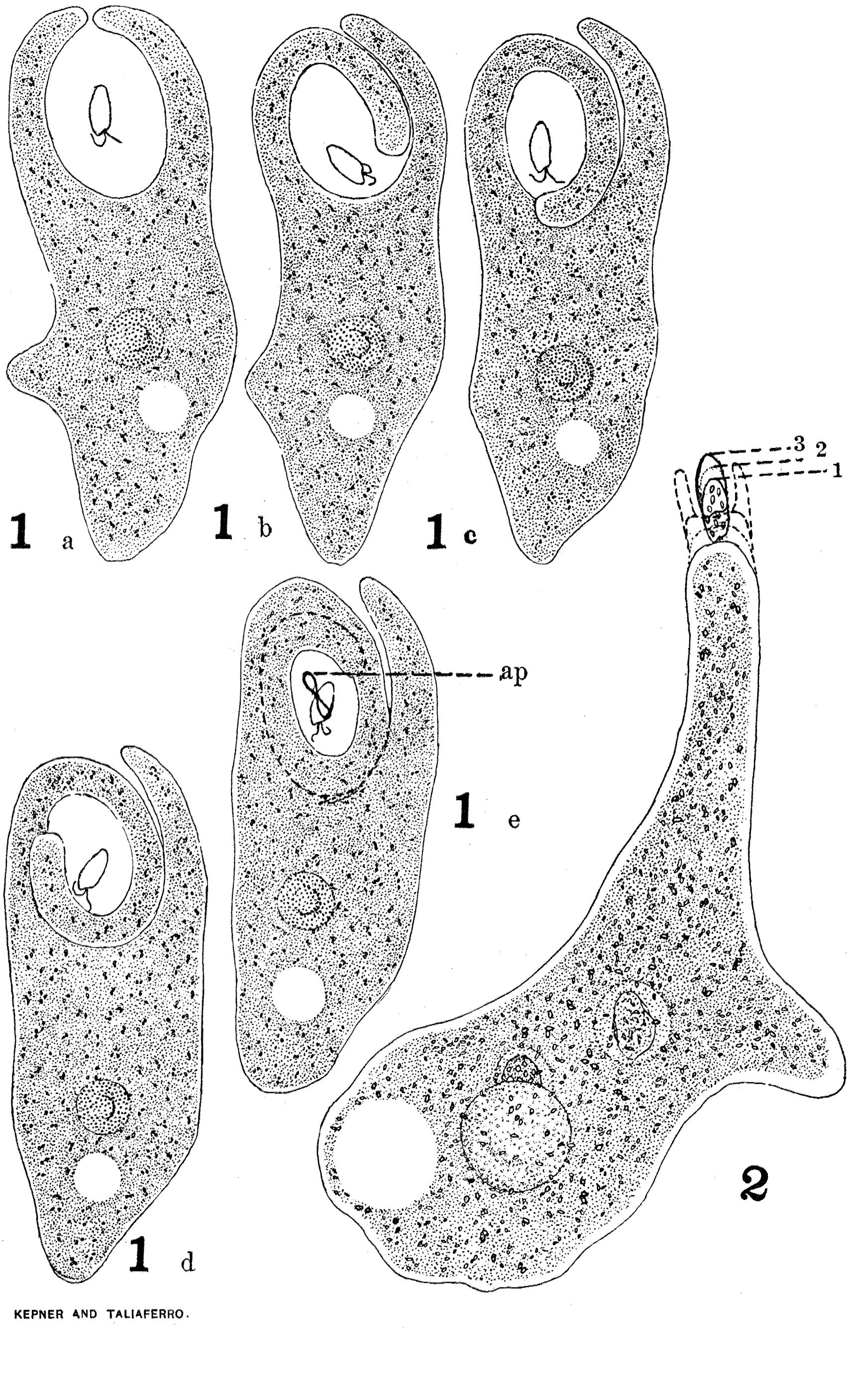
Explanation of Plate I.

Fig. 1. (*a*) The pseudopodia have closed behind the prey; (*b*), (*c*), (*d*), and (*e*) shows how the left pseudopodium grew down along and then away from the right pseudopodium. The broken line in (*e*) indicates the place where the two surfaces of the left pseudopodium fused to enclose the prey. (*ap*) indicates the bilobed margin of the ectoplasm that over-arched the forming food vacuole. ×166.

Fig. 2. The *Chilomonas paramœcium* was first encountered at position 1. The prey next retreated to position 2, indicated by the dotted contour. When the second contact with the *Amœba proteus* was made it retreated to position 3. The contour of the position 3 is indicated by the darkest line. It lay in this third position when it was ingested by the *Amœba*. ×333.


1 a
1 b
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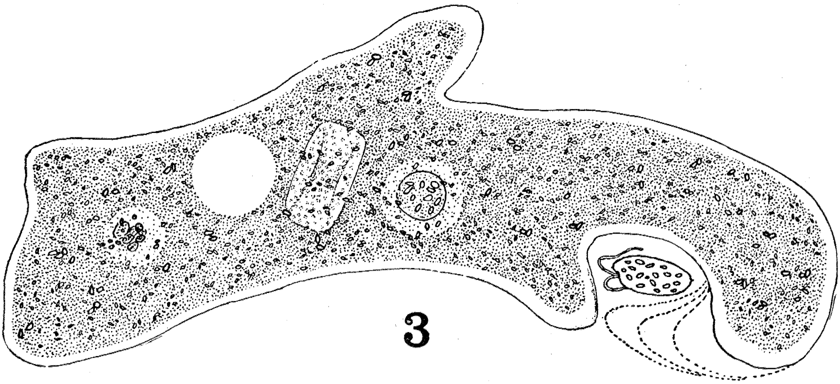
Explanation of Plate II.

FIG. 3. Shows a *Chilomonas* lying between a lateral and a terminal pseudopodium. The response to the contact or contacts made by the *Chilomonas* was the lateral and posterior growth of only the terminal pseudopodium. The successive steps in the advance of this pseudopodium is indicated by the broken lines. ×333.

FIG. 4. The *Chilomonas* by advancing and retreating made three contacts at *a*. The response of the *Amœba* to these contacts was not at the point stimulated but at points (*b*) and (*c*). From these points secondary pseudopodia grew towards each other and fused behind the prey, as indicated by the dotted lines. ×333.

FIG. 5. A *Chilomonas* entered the wide angle between the two large pseudopodia in the direction indicated by the arrow. When it met the *Amœba* it lay in contact with the ectoplasm. In response to this contact a small secondary pseudopodium (*b*) arose behind the prey and a large pseudopodium (*a*) arose from the apex of the inter-pseudopodial angle. ×333.



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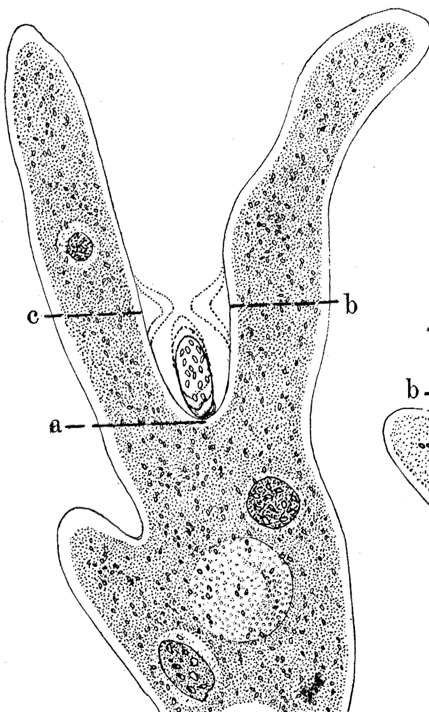

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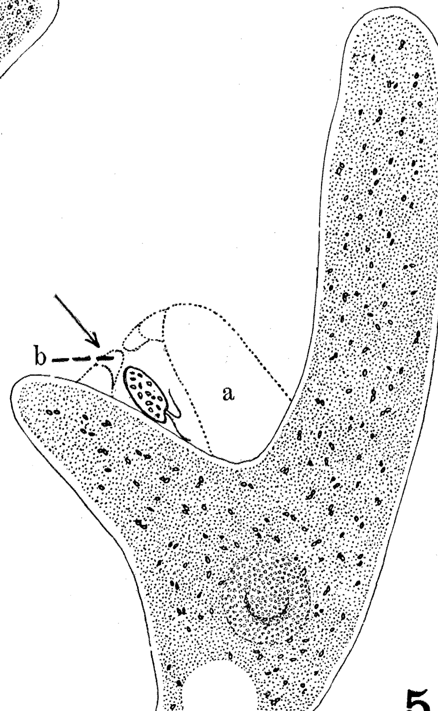

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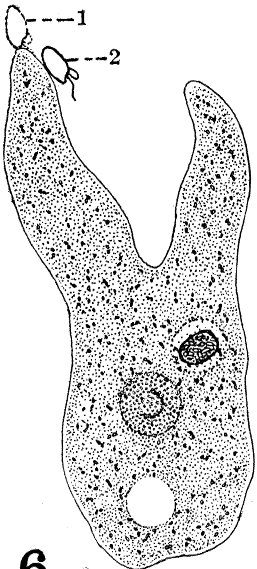
Explanation of Plate III.

FIG. 6. (*a*) The *Chilomonas* first lay at the apex of the pseudopodium. As the pseudopodium advanced it glided by the *Chilomonas* so that the latter lay to the right in contact with the ectoplasm at the point indicated by position 2. (*b*), (*c*), and (*d*) show the wave-like character of the reaction. This reaction crowded or dragged the passive prey down into the fundus of the interpseudopodial space where the *Chilomonas* was enclosed in a vacuole formed by the fusion of the apex of the lateral active pseudopodium and the base of the right, inactive pseudopodium. ×166.

FIG. 7. The large pseudopodium of *Amœba* came in contact at its apex with the *Chilomonas* (1). The *Chilomonas* was then pushed to position (2). The pseudopodium next advanced beyond the prey and turned to the right at (*a*) along its upper side. At the same time a secondary pseudopodium was thrown out at (*b*). Later this reaction on the part of the *Amœba* was changed; the pseudopodium at (*b*) was withdrawn and one thrown out at (*c*) to better meet the conditions necessary for the capture of the prey. Pseudopodia (*a*) and (*c*) then grew around the *Chilomonas* and enclosed it in a food vacuole. ×333.



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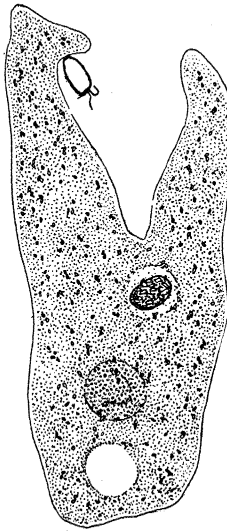

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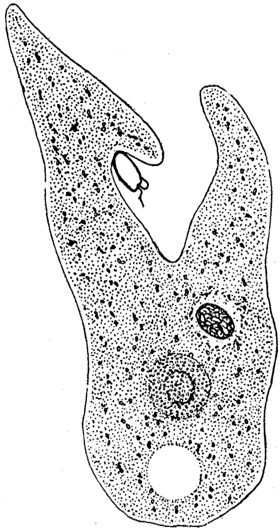

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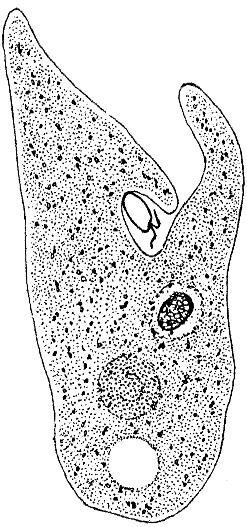

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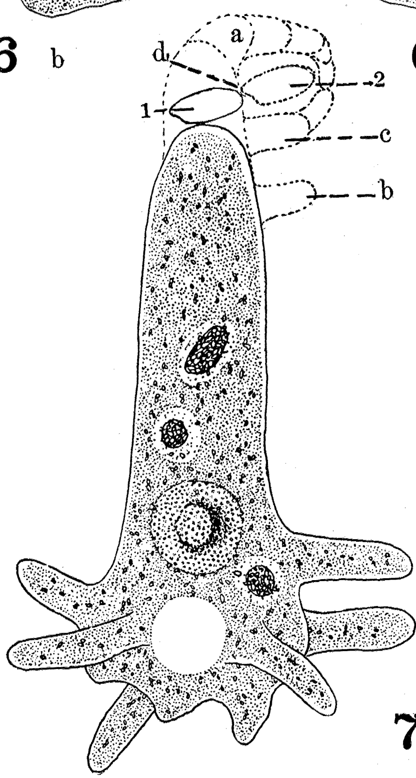

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